

Northumbria Research Link

Citation: Hill, Rebecca M, Richardson, Stacey, Schwalbe, Ed, Hicks, Debbie, Lindsey, Janet C, Crosier, Stephen, Rafiee, Gholamreza, Grabovska, Yura, Wharton, Stephen B, Jacques, Thomas S, Michalski, Antony, Joshi, Abhijit, Pizer, Barry, Williamson, Daniel, Bailey, Simon and Clifford, Steven C (2020) Time, pattern, and outcome of medulloblastoma relapse and their association with tumour biology at diagnosis and therapy: a multicentre cohort study. *The Lancet Child & Adolescent Health*, 4 (12). pp. 865-874. ISSN 2352-4642

Published by: Elsevier

URL: [https://doi.org/10.1016/S2352-4642\(20\)30246-7](https://doi.org/10.1016/S2352-4642(20)30246-7) <[https://doi.org/10.1016/S2352-4642\(20\)30246-7](https://doi.org/10.1016/S2352-4642(20)30246-7)>

This version was downloaded from Northumbria Research Link:
<http://nrl.northumbria.ac.uk/id/eprint/44606/>

Northumbria University has developed Northumbria Research Link (NRL) to enable users to access the University's research output. Copyright © and moral rights for items on NRL are retained by the individual author(s) and/or other copyright owners. Single copies of full items can be reproduced, displayed or performed, and given to third parties in any format or medium for personal research or study, educational, or not-for-profit purposes without prior permission or charge, provided the authors, title and full bibliographic details are given, as well as a hyperlink and/or URL to the original metadata page. The content must not be changed in any way. Full items must not be sold commercially in any format or medium without formal permission of the copyright holder. The full policy is available online: <http://nrl.northumbria.ac.uk/policies.html>

This document may differ from the final, published version of the research and has been made available online in accordance with publisher policies. To read and/or cite from the published version of the research, please visit the publisher's website (a subscription may be required.)



**Northumbria
University**
NEWCASTLE



UniversityLibrary

Time, pattern, and outcome of medulloblastoma relapse and their association with tumour biology at diagnosis and therapy: a multicentre cohort study



Rebecca M Hill, Stacey Richardson, Edward C Schwalbe, Debbie Hicks, Janet C Lindsey, Stephen Crosier, Gholamreza Rafiee, Yura Grabovska, Stephen B Wharton, Thomas S Jacques, Antony Michalski, Abhijit Joshi, Barry Pizer, Daniel Williamson, Simon Bailey, Steven C Clifford



Summary

Background Disease relapse occurs in around 30% of children with medulloblastoma, and is almost universally fatal. We aimed to establish whether the clinical and molecular characteristics of the disease at diagnosis are associated with the nature of relapse and subsequent disease course, and whether these associations could inform clinical management.

Methods In this multicentre cohort study we comprehensively surveyed the clinical features of medulloblastoma relapse (time to relapse, pattern of relapse, time from relapse to death, and overall outcome) in centrally reviewed patients who relapsed following standard upfront therapies, from 16 UK Children's Cancer and Leukaemia Group institutions and four collaborating centres. We compared these relapse-associated features with clinical and molecular features at diagnosis, including established and recently described molecular features, prognostic factors, and treatment at diagnosis and relapse.

Findings 247 patients (175 [71%] boys and 72 [29%] girls) with medulloblastoma relapse (median year of diagnosis 2000 [IQR 1995–2006]) were included in this study. 17 patients were later excluded from further analyses because they did not meet the age and treatment criteria for inclusion. Patients who received upfront craniospinal irradiation (irradiated group; 178 [72%] patients) had a more prolonged time to relapse compared with patients who did not receive upfront craniospinal irradiation (non-irradiated group; 52 [21%] patients; $p < 0.0001$). In the non-irradiated group, craniospinal irradiation at relapse (hazard ratio [HR] 0.27, 95% CI 0.11–0.68) and desmoplastic/nodular histology (0.23, 0.07–0.77) were associated with prolonged time to death after relapse, *MYC* amplification was associated with a reduced overall survival (23.52, 4.85–114.05), and re-resection at relapse was associated with longer overall survival (0.17, 0.05–0.57). In the irradiated group, patients with MB_{Group3} tumours relapsed significantly more quickly than did patients with MB_{Group4} tumours (median 1.34 [0.99–1.89] years vs 2.04 [1.39–3.42] years; $p = 0.0043$). Distant disease was prevalent in patients with MB_{Group3} (23 [92%] of 25 patients) and MB_{Group4} (56 [90%] of 62 patients) tumour relapses. Patients with distantly-relapsed MB_{Group3} and MB_{Group4} displayed both nodular and diffuse patterns of disease whereas isolated nodular relapses were rare in distantly-relapsed MB_{SHH} (1 [8%] of 12 distantly-relapsed MB_{SHH} were nodular alone compared with 26 [34%] of 77 distantly-relapsed MB_{Group3} and MB_{Group4}). In MB_{Group3} and MB_{Group4} nodular disease was associated with a prolonged survival after relapse (HR 0.42, 0.21–0.81). Investigation of second-generation MB_{Group3} and MB_{Group4} molecular subtypes refined our understanding of heterogeneous relapse characteristics. Subtype VIII had prolonged time to relapse and subtype II had a rapid time from relapse to death. Subtypes II, III, and VIII developed a significantly higher incidence of distant disease at relapse whereas subtypes V and VII did not (equivalent rates to diagnosis).

Interpretation This study suggests that the nature and outcome of medulloblastoma relapse are biology and therapy-dependent, providing translational opportunities for improved disease management through biology-directed disease surveillance, post-relapse prognostication, and risk-stratified selection of second-line treatment strategies.

Funding Cancer Research UK, Action Medical Research, The Tom Grahame Trust, The JGW Patterson Foundation, Star for Harris, The Institute of Child Health - Newcastle University - Institute of Child Health High-Risk Childhood Brain Tumour Network (co-funded by The Brain Tumour Charity, Great Ormond Street Children's Charity, and Children with Cancer UK).

Copyright © 2020 The Author(s). Published by Elsevier Ltd. This is an Open Access article under the CC BY 4.0 license.

Introduction

Over the past decade, advances have been made in the understanding of medulloblastoma biology at diagnosis. Four consensus molecular subgroups—WNT (MB_{WNT}),

SHH (MB_{SHH}), group 3 (MB_{Group3}), and group 4 (MB_{Group4})—are now recognised and underpin the 2016 WHO classification of medulloblastoma (genetically defined as WNT-activated; SHH-activated and *TP53*-mutant and

Lancet Child Adolesc Health
2020; 4: 865–74

Published Online
October 22, 2020
[https://doi.org/10.1016/S2352-4642\(20\)30246-7](https://doi.org/10.1016/S2352-4642(20)30246-7)

Wolfson Childhood Cancer Research Centre, Newcastle University Centre for Cancer, Newcastle upon Tyne, UK (R M Hill PhD, S Richardson PhD, E C Schwalbe PhD, D Hicks PhD, J C Lindsey PhD, S Crosier PhD, G Rafiee PhD, Y Grabovska PhD, D Williamson PhD, Prof S Bailey PhD, Prof S C Clifford PhD); Department of Applied Sciences, Northumbria University, Newcastle upon Tyne, UK (E C Schwalbe); School of Electronics, Electrical Engineering and Computer Science, Queen's University Belfast, Centre for Cancer Research & Cell Biology, UK (G Rafiee); Sheffield Institute for Translational Neuroscience, University of Sheffield, Sheffield, UK (Prof S B Wharton MD); Neural Development Unit, UCL Institute of Child Health, London, UK (Prof T S Jacques MD, A Michalski MD); Department of Neuropathology, Royal Victoria Infirmary, Newcastle University Teaching Hospitals, NHS Foundation Trust, Newcastle upon Tyne, UK (A Joshi MD); and Institute of Translational Research, University of Liverpool, Liverpool, UK (Prof B Pizer MD)

Correspondence to:
Prof Steven C Clifford, Wolfson Childhood Cancer Research Centre, Newcastle University Centre for Cancer, Newcastle upon Tyne NE1 7RU, UK
steve.clifford@ncl.ac.uk

Research in context

Evidence before this study

Relapse after conventional upfront therapies is the most adverse prognostic factor for childhood medulloblastoma. However, associations between molecular pathology at diagnosis and relapse characteristics are not well understood. We searched PubMed for articles published between Jan 1, 1990, and Dec 31, 2019, with the search terms “medulloblastoma”, and “relapse OR recurrence”. Several clinical and biology studies were identified and reviewed. Two initial reports have highlighted the clinical potential in such studies. The first investigated molecular subgroup at diagnosis in three independent cohorts (n=30, 77, and 96), and delineated subgroup-specific differences in relapse patterns and timings. However, findings showed variability between cohorts, and this study did not consider clinical and molecular features other than subgroup, treatment-related differences, or the radiological patterns of distantly-relapsed tumours. The second reported a retrospective analysis of a European trial for standard-risk medulloblastoma (HIT-SIOP-PNET4; completion 2006), describing in detail the patterns of disease relapse in all patients who relapsed (n=72). However, biological annotation at diagnosis was limited and this trial did not include patients at a high risk of relapse.

Added value of this study

Systematic assessment of the nature of medulloblastoma relapse, and its relationship to established and novel clinical

and molecular features assessable at diagnosis and treatment received is essential to establish any evidence base for altered clinical practice. We report comprehensive investigation of the molecular, clinical, and radiological features, and the inter-relationships, of a centrally reviewed cohort of 247 children with medulloblastoma, who relapsed after conventional upfront therapies. These are estimated to represent the relapsing component of over 800 patients, approximately double the size of the largest reported international medulloblastoma clinical trials.

Implications of all the available evidence

Our study shows the associations between the clinical and molecular characteristics of medulloblastoma at diagnosis, treatment received, and the nature of relapse and subsequent post-relapse disease course. The scale, clinical orientation, and systematic nature of our study support and challenge specific conclusions from previous studies and has uncovered new relationships. Together, these findings provide an evidence base to support translational opportunities aimed at improved disease management and outcomes, through biology-directed disease surveillance, post-relapse prognostication, and risk-stratified selection of second-line treatment strategies.

SHH-activated and *TP53*-wildtype; and non-WNT and non-SHH [group3 and group4]).¹ Molecular profiling of a greater number of tumours at diagnosis has led to the discovery of second-generation MB_{Group3} and MB_{Group4} subtypes.^{2,3} Furthermore, molecular features have prognostic significance and define upfront therapy stratification. For example, molecular features identified in international clinical trials (eg, SJMB12 [NCT01878617] and SIOP-PNET5-MB [NCT02066220]) have led to therapy reduction in groups with a good prognosis (eg, MB_{WNT}) and treatment intensification or the use of novel therapies for groups at a high risk of relapse and mortality (eg, *MYC*-amplified MB_{Group3}).

Disease relapse is the most adverse prognostic factor in medulloblastoma. Relapses occur in approximately 30% of patients and are almost always fatal.^{4–6} Most patients will relapse at distant CNS sites with or without disease in the original tumour bed. Individual reports indicate relapse can occur more than 5 years after diagnosis, with the duration of survival after relapse equally as variable.^{3–5,7} Durable remissions following relapse typically cluster in younger children (aged <3–5 years) who received deferred craniospinal irradiation as salvage therapy.^{8,9} Older children (aged >3–5 years) with disease relapse who received conventional upfront therapy (neurosurgery, craniospinal irradiation, and chemotherapy) are treated with various strategies at relapse, including metronomic

therapy, high-dose chemotherapy, intrathecal medication, and re-irradiation, but these approaches are commonly unsuccessful (typically <5% long-term survival).^{6,7,10–13} Of note, few studies have attempted to characterise the nature of medulloblastoma relapse, its clinical and molecular correlates, and their translational potential.^{5,7}

Here, we report the nature of medulloblastoma relapse (ie, timings, radiological patterns, and post-relapse outcomes), and its associations with treatment and both established and contemporary clinical and molecular features, in a cohort of patients with medulloblastoma who relapsed. This study encompasses all established disease-wide molecular features and second-generation MB_{Group3} and MB_{Group4} subtypes.^{2,3}

Methods

Study design and participants

In this multicentre cohort study, patients aged 0–18 years at diagnosis with medulloblastoma who had relapsed following upfront therapy were assembled from 16 UK Children's Cancer and Leukaemia Group institutions and four collaborating centres. Eligible patients had a confirmed diagnosis of medulloblastoma, a defined period of remission following upfront therapy, and a documented relapse on imaging or biopsy. According to current treatment conventions, infants (aged <3–5 years, cutoff depending on national treatment protocols)

commonly received radiotherapy-sparing treatment at diagnosis; whereas children (aged >3–5 years) received conventional multimodal therapy, which included craniospinal irradiation.¹⁴ Therefore, we distinguished two restricted subcohorts for specific analyses: patients older than 3 years who received craniospinal irradiation at diagnosis (irradiated group) and patients younger than 5 years who did not receive craniospinal irradiation (non-irradiated group).

Pathological variant was assigned according to 2016 WHO criteria.¹ Metastatic status at diagnosis was determined according to Chang's criteria.¹⁵ All patients in the restricted irradiated group received conventional radiotherapy protocols (mode 36/54 Gy; range 24–40/50–60 Gy) following maximal safe neurosurgical resection. In the non-irradiated group, some patients received focal radiotherapy (mode 54 Gy; range 45–55 Gy). Institutional imaging reports were collated and centrally reviewed by an experienced panel of paediatric neuro-oncologists (RMH and SB) to assess the degree of surgical resection (subtotal resection or gross total resection) and patterns of relapse. When interpretation of reported patterns was unclear, clarification was sought with the originating centre or clinical team. Patients with nodular relapses (defined as single or multiple discrete lesions) were distinguished from patients with diffuse (ie, diffuse leptomeningeal dissemination) or mixed-pattern relapses. Similarly, patients with local relapses were considered separately from patients with either distant or combined local and distant relapses. All patients had a documented clinical remission on imaging before relapse.¹⁶

Procedures

Established disease-wide molecular features either accepted in the WHO classification of medulloblastoma, or used as the current basis for treatment stratification, were evaluated.¹ These comprised molecular subgroup (MB_{WNT}, MB_{SHH}, MB_{Group3}, and MB_{Group4}), *TP53* mutation status, and *MYC* or *MYCN* oncogene amplification.¹⁷ Additionally, second-generation methylation subtypes, isochromosome 17q, and *TERT* mutations were evaluated as additional features with potential clinical relevance.^{2,3,18–20}

Tumours were assigned to consensus molecular subgroups, as previously described using non-negative matrix factorisation and t-distributed stochastic neighbor embedding with density-based spatial clustering of applications with noise clustering.²¹ For samples with a low yield of DNA, the minimal methylation classifier was used.²² For MB_{Group3} and MB_{Group4} in the irradiated group, second-generation high-risk and low-risk subgroups were assigned as previously described,² and subtypes I–VIII were identified according to the Group 3 and Group 4 classifier.³ *TP53* mutation status was assessed for exons 4–9 using established methods.⁴ Copy-number assessment by interphase fluorescent in-situ hybridisation of *MYC* (8q24–21 probes), *MYCN*

	Entire cohort (n=247)	Irradiated group (n=178)	Non-irradiated group (n=52)
Patterns of relapse			
Nodular	86/187 (46%)	53/125 (42%)	23/47 (49%)
Diffuse	66/187 (35%)	46/125 (37%)	17/47 (36%)
Nodular and diffuse	35/187 (19%)	26/125 (21%)	7/47 (15%)
Local relapse	43/193 (22%)	21/129 (16%)	16/48 (33%)
Distant relapse	96/193 (50%)	73/129 (57%)	16/48 (33%)
Combined relapse	54/193 (28%)	35/129 (27%)	16/48 (33%)
Clinical features at disease relapse			
Boys	175/247 (71%)	133/178 (75%)	32/52 (62%)
Girls	72/247 (29%)	45/178 (25%)	20/52 (38%)
Boys:girls ratio	2.4:1	3:1	1.6:1
Infants (<4.0 years)	38/247 (15%)	1/178 (1%)	32/52 (62%)
Children (4.0–16.0 years)	176/247 (72%)	145/178 (81%)	20/52 (38%)
Adults (>16.0 years)	33/247 (13%)	32/178 (18%)	0/52
Treatment at disease relapse			
Resection	47/182 (26%)	32/124 (26%)	12/45 (27%)
Chemotherapy	128/185 (69%)	102/127 (80%)	22/46 (48%)
Craniospinal irradiation	19/188 (10%)	1/127 (1%)	15/48 (31%)
Focal radiotherapy	23/188 (12%)	18/127 (14%)	4/48 (8%)
Clinicopathological features and treatment at diagnosis			
Infants (<4.0 years)	67/247 (27%)	11/178 (6%)	48/52 (92%)
Children (4.0–16.0 years)	172/247 (70%)	160/178 (90%)	4/52 (8%)
Adults (>16.0 years)	8/247 (3%)	7/178 (4%)	0/52
Subtotal resection	78/245 (32%)	58/178 (33%)	14/52 (27%)
Chemotherapy	206/243 (84%)	142/177 (80%)	49/50 (98%)
Craniospinal irradiation	187/247 (76%)	178/178 (100%)	0/178
Classic histology	158/226 (70%)	123/162 (76%)	28/48 (58%)
Large-cell anaplastic histology	39/226 (17%)	26/162 (16%)	8/48 (17%)
Desmoplastic/nodular histology	29/226 (13%)	13/162 (8%)	12/48 (25%)
Distant disease	77/238 (32%)	57/170 (34%)	13/52 (25%)
Established molecular features at diagnosis			
<i>MYC</i> amplification	13/220 (6%)	5/157 (3%)	6/48 (13%)
<i>MYCN</i> amplification	24/217 (11%)	20/155 (13%)	2/47 (4%)
<i>TP53</i> mutation	19/216 (9%)	18/150 (12%)	0/49
Isochromosome 17q	50/167 (30%)	40/117 (34%)	6/38 (16%)
<i>TERT</i> mutation	14/221 (6%)	10/162 (6%)	2/44 (5%)
MB _{WNT}	5/216 (2%)	5/156 (3%)	0/44
MB _{SHH}	60/216 (28%)	29/156 (19%)	25/44 (57%)
MB _{Group3}	62/216 (29%)	40/156 (26%)	15/44 (34%)
MB _{Group4}	89/216 (41%)	82/156 (53%)	4/44 (9%)

Data are n/N (%). MB=medulloblastoma. 17 patients from the entire cohort were excluded because they did not meet the restricted cohort criteria.

Table 1: Patterns of relapse and clinical and molecular features of the entire cohort and the two age-restricted subcohorts

(2p24.3 probes), and chromosome 17 imbalances (17p13.3 and 17q12 probes) was done as previously described.⁴ *TERT* promoter mutation detection (mutations 228C→T and 250C→T) was done according to previous reports.¹⁹ An additional locked nucleic acid probe was designed to detect the 228C→A mutation

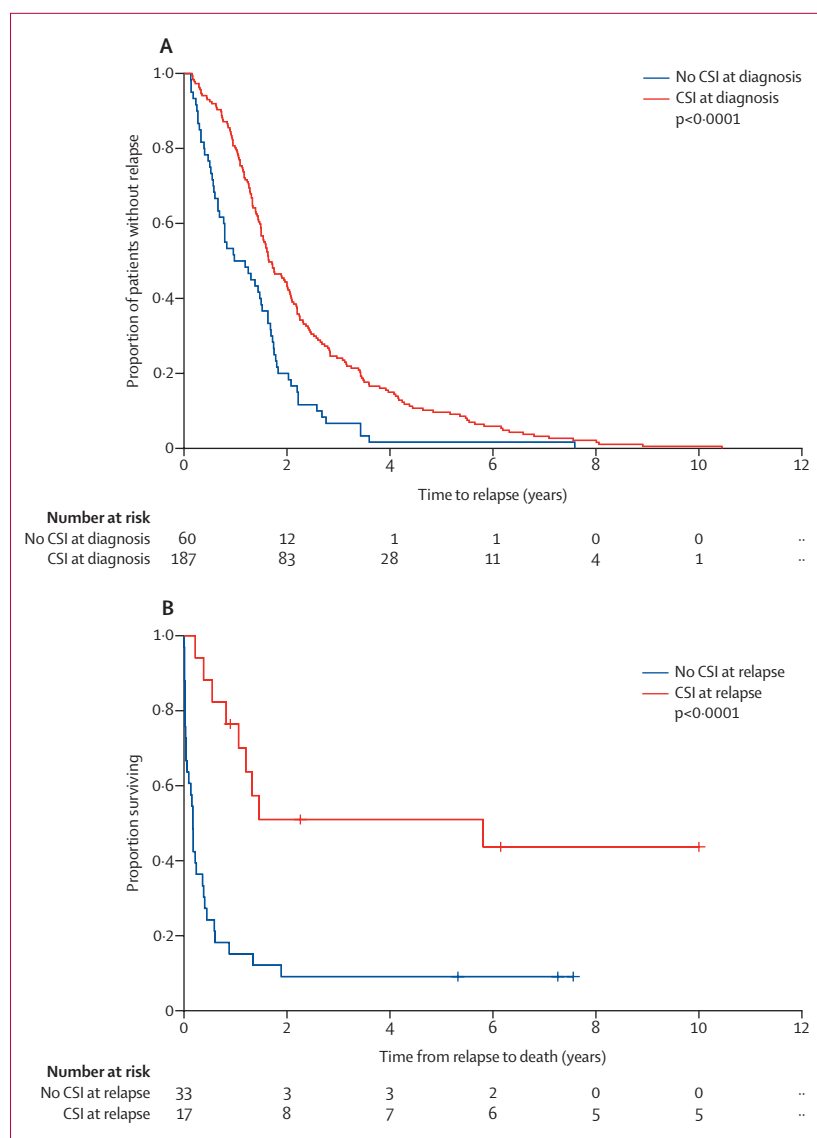


Figure 1: Time to relapse and survival after medulloblastoma relapse according to treatment received (A) Time to relapse according to whether CSI was delivered at diagnosis. (B) Time from relapse to death or last follow-up in patients who did not receive upfront CSI, according to whether CSI was delivered at relapse. Ten patients were removed from time from relapse to death analysis due to: death from other cause (n=1), alive with disease (n=5), or missing data (n=4). CSI=craniospinal irradiation.

(*TERT* mut 228C→A, 5'-56FAM-CCC CAT CCG G-3IABkFQ-3'; Integrated DNA Technologies, Coralville, IO, USA). Genotyping for this mutation was done separately with a probe targeted to the wild type sequence (*TERT* WT, 5'-5HEX-CCC CTC CCG G-3IABkFQ-3'; Integrated DNA Technologies, Coralville, IO, USA) using the same method.

Statistical analysis

Individual missing datapoints were missing completely at random. All recurrently detected (ie, occurred twice or more) established features were tested. Second-generation MB_{Group3} and MB_{Group4} methylation subtypes which were

present in more than 10% of patients with MB_{Group3} and MB_{Group4} tumours treated with craniospinal irradiation, were also included for subanalyses. χ^2 and Fisher's exact tests were used to assess associations between clinical and molecular features. The log-rank test was used in univariable analyses to assess time to relapse, time from relapse to death, and overall survival, and the Kaplan-Meier method was used to visualise results. Cox proportional-hazards models were used to investigate the significance of all covariates for time to relapse, time to death after relapse, and overall survival in univariable and multivariable models, using forward likelihood-ratio testing. In the time to death and overall survival analyses, data were censored for patients who died of other causes or were alive with disease. We tested the proportionality assumption for Cox modelling using scaled Schoenfeld residuals. Proportional covariates with a raw p value of less than 0.1 in univariable analyses were taken forward as candidates for multivariable modelling. Multivariable models were restricted to three covariates in the entire cohort, and two covariates for all subcohorts. The Benjamini-Hochberg procedure was used to control the false discovery rate and adjusted p values of less than 0.05 identified significant associations. Analysis and visualisation was done using GraphPad Prism (version 8.4.1) and the R statistical environment (version 3.5.3).

Role of the funding source

The funders had no role in study design, cohort collection, analyses, data interpretation, or writing of the report. RMH, ECS, GR, DW, and SCC had access to raw data. The corresponding author had full access to all data and had final responsibility for the decision to submit for publication.

Results

247 patients (175 [71%] boys and 72 [29%] girls) with medulloblastoma relapse were included in the study (table 1). 17 patients were later excluded from analyses because they did not meet the age and treatment criteria for inclusion. 34 (15%) patients had tumour samples collected in 2010–14, 87 (37%) in 2000–09, 90 (39%) in 1990–99, and the remaining 21 (9%) with data available were collected before 1990. Year of diagnosis was unavailable in 15 samples. All variables were assessed by era (before 2000 vs 2000 and after). There were no variables that biased planned analyses (appendix pp 2–4). As expected, age and upfront treatment were confounded ($p<0.0001$; appendix p 5). Pathological variant was centrally assessed in 222 (90%) patients, local assessment was done for the remaining 25 (10%) participants (table 1).

We first characterised relapse-specific features—including patterns of relapse (eg, nodular or diffuse disease) and treatment history—and established disease features at diagnosis in the entire cohort (table 1) and assessed these against time to relapse and overall survival.

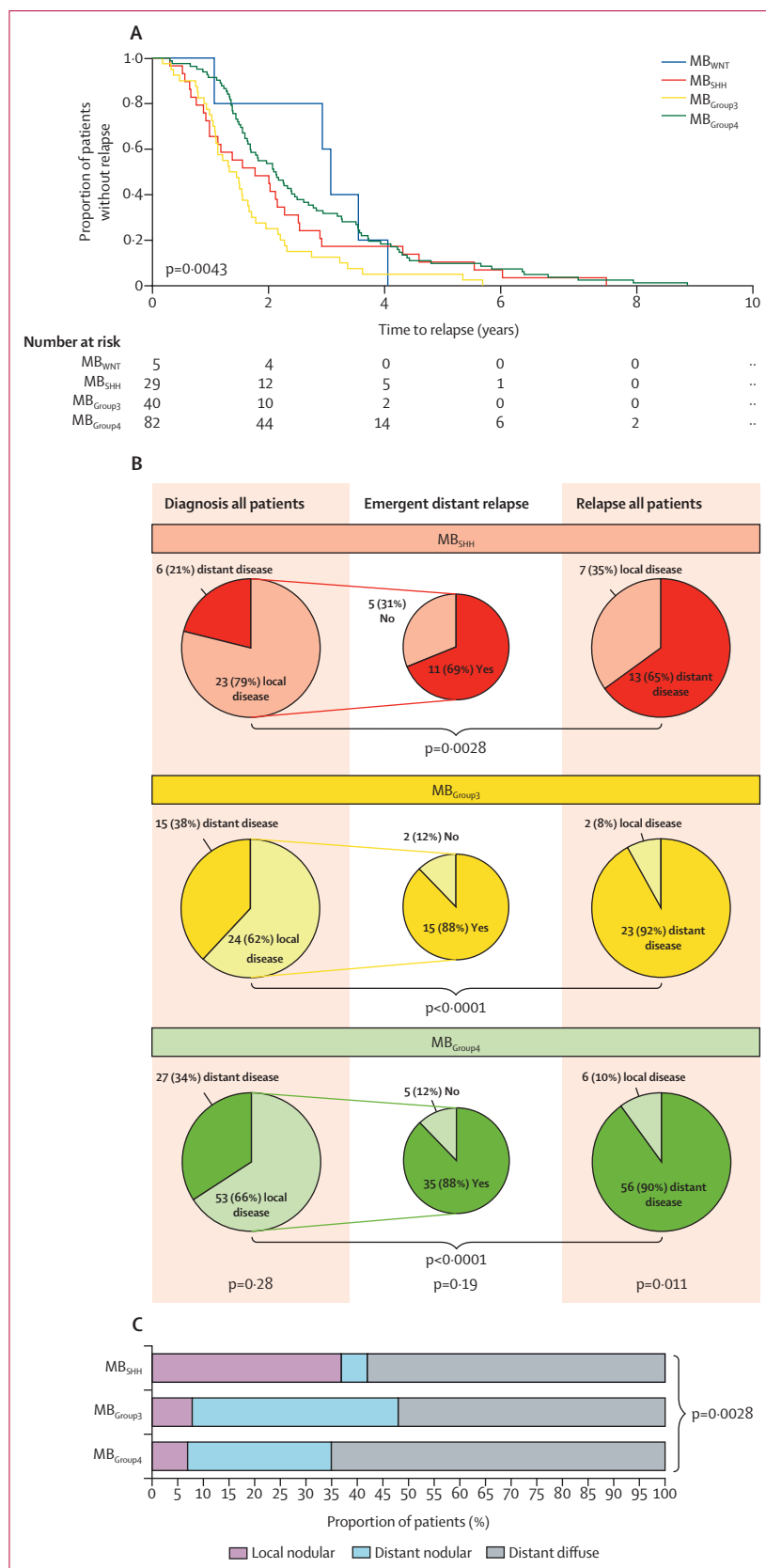
	Frequency	Univariable analyses				Multivariable analyses			
		Time to relapse		Overall survival		Time to relapse		Overall survival	
		HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value
Patterns of disease relapse									
Nodular	86/187 (46%)	0.70 (0.51–0.97)	0.096	0.61 (0.42–0.89)	0.010
Distant relapse	150/193 (78%)	1.09 (0.74–1.62)	0.78
Treatment at disease relapse									
Resection	47/182 (26%)	0.50 (0.34–0.74)	0.0026
Chemotherapy	128/185 (69%)	0.70 (0.50–0.99)	0.12
Craniospinal irradiation	19/188 (10%)	0.29 (0.15–0.58)	0.0024
Focal radiotherapy	23/188 (12%)	0.64 (0.36–1.14)	0.27
Any radiotherapy	42/188 (22%)	0.39 (0.25–0.62)	0.00057	0.40 (0.24–0.67)	0.00052
Clinicopathological features and treatment at diagnosis									
Boys	175/247 (71%)	0.92 (0.70–1.22)	0.68	0.10 (0.74–1.35)	0.98
Infants (<4 years)	67/247 (27%)	1.32 (0.99–1.75)	0.15	0.84 (0.61–1.17)	0.52
Subtotal resection	78/245 (32%)	1.01 (0.77–1.32)	0.95	1.35 (1.00–1.81)	0.11
Chemotherapy	206/243 (85%)	1.15 (0.80–1.64)	0.65	1.08 (0.74–1.58)	0.77
Craniospinal irradiation	187/247 (76%)	0.52 (0.39–0.70)	0.00011	0.95 (0.68–1.34)	0.81
Focal radiotherapy	18/247 (7%)	1.05 (0.65–1.70)	0.88	1.33 (0.76–2.35)	0.50
Any radiotherapy	205/247 (83%)	0.38 (0.27–0.54)	<0.0001	1.06 (0.72–1.58)	0.83	0.34 (0.23–0.50)	<0.0001
Classic histology	158/226 (70%)	0.69 (0.52–0.92)	0.030	0.89 (0.65–1.22)	0.62
Large-cell anaplastic histology	39/226 (17%)	1.64 (1.16–2.33)	0.018	2.17 (1.49–3.17)	0.00083	1.62(1.12–2.34)	0.0097
Desmoplastic/nodular histology	29/226 (13%)	1.13 (0.76–1.67)	0.69	0.57 (0.36–0.91)	0.075
Distant disease	77/238 (32%)	1.19 (0.90–1.56)	0.44	1.08 (0.80–1.45)	0.76
Established molecular features at diagnosis									
MYC amplification	13/220 (6%)	3.80 (2.15–6.72)	<0.0001	5.39 (3.01–9.62)	<0.0001	6.18 (3.17–12.05)	<0.0001
MYCN amplification	24/217 (11%)	1.27 (0.83–1.94)	0.50	1.69 (1.07–2.68)	0.082
TP53 mutation	19/216 (9%)	1.22 (0.76–1.96)	0.65	1.3 (0.79–2.12)	0.54
Isochromosome 17q	50/167 (30%)	0.82 (0.48–1.42)	0.70	1.17 (0.82–1.68)	0.57
TERT mutation	14/221 (6%)	0.92 (0.66–1.29)	0.66	1.17 (0.68–2.02)	0.73
MB _{WNT}	5/216 (2%)	0.66 (0.27–1.60)	0.59	0.56 (0.14–2.27)	0.59
MB _{SHH}	60/216 (28%)	1.32 (0.98–1.78)	0.15	0.82 (0.58–1.16)	0.50
MB _{Group3}	62/216 (29%)	1.82 (1.35–2.46)	0.00041	1.92 (1.40–2.65)	0.00044	1.83 (1.33–2.54)	0.00024
MB _{Group4}	89/216 (41%)	0.55 (0.41–0.72)	0.00011	0.75 (0.55–1.00)	0.12
HR=hazard ratio. MB=medulloblastoma.									
Table 2: Univariable and multivariable analyses of correlates of time to relapse and overall survival in the entire cohort									

Table 2: Univariable and multivariable analyses of correlates of time to relapse and overall survival in the entire cohort

In univariable analyses of the entire cohort, upfront craniospinal irradiation significantly prolonged time to relapse ($p<0.0001$; figure 1A). Similarly, craniospinal irradiation at relapse was associated with an improved overall survival ($p=0.0024$; table 2). In 60 patients who did not receive upfront craniospinal irradiation, craniospinal irradiation at relapse was associated with survival after relapse ($p<0.0001$; figure 1B). Multivariable Cox modelling showed that any radiotherapy at diagnosis significantly increased time to relapse; whereas large-cell anaplastic histology and MB_{Group3} tumour subgroup significantly decreased time to relapse. In the entire cohort, nodular relapses and radiotherapy at relapse significantly increased overall survival and MYC amplification significantly decreased overall survival (table 2).

Considering these radiotherapy-associated survival differences and the established association between age at diagnosis and upfront craniospinal irradiation,²³ we divided the cohort into two groups: 178 children were included in the irradiated group and 52 children were included in the non-irradiated group (table 1). All subsequent analyses were done independently within these two restricted groups.

In univariable survival analyses of the irradiated group across all time-based measures, patients with MB_{Group3} tumours had a shorter time to relapse (median 1.34 years [IQR 0.99–1.89]) than did those with MB_{Group4} tumours (2.04 years [1.39–3.42]; $p=0.0043$; figure 2A). Overall, 18 (10%) of 178 patients in the irradiated group relapsed after 5 years surveillance; of 13 patients with



available molecular subgroup data, eight (62%) had MB_{Group4} tumours. Of note, no other feature examined in univariable analyses, including molecular features, such as *MYCN* amplification and *TP53* mutation at diagnosis, were associated with a more rapid disease course (table 3). In multivariable Cox modelling, MB_{Group3} and *MYC* amplification were independent adverse prognostic factors for time to relapse (table 3). Large-cell anaplastic histology and *MYC* amplification were independent adverse risk factors for overall survival. Large-cell anaplastic histology was associated with reduced time to death from relapse (appendix p 6). Of note, molecular subgroup was not associated with time to death from relapse (appendix p 6).

In univariable survival analyses of the non-irradiated group, high-risk features (isochromosome 17q, large-cell anaplastic histology, and *MYC* amplification) were associated with a more rapid time to relapse and subsequent poorer overall survival (appendix p 7). In univariable analyses, desmoplastic/nodular histology, MB_{SHH} tumours, and craniospinal irradiation at relapse were associated with a sustained overall survival (appendix p 7). In multivariable Cox modelling, *MYC* amplification and large-cell anaplastic histology (appendix p 7) were significant independent risk factors for reduced time to relapse whereas desmoplastic/nodular histology (hazard ratio [HR] 0.23, 95% CI 0.07–0.77) and craniospinal irradiation at relapse were significantly associated with increased time to death after relapse (0.27, 0.11–0.68; appendix p 7). *MYC* amplification was a significant independent risk factor for decreased overall survival (23.52, 4.85–114.05); however, resection at relapse was associated with longer overall survival (0.17, 0.05–0.57; appendix p 7).

Nine (5%) of 178 patients in the irradiated group survived their relapse. Median follow-up after relapse was 4.72 years (IQR 3.54–10.15), with no common factors observed in these survivors (appendix p 8). 11 (21%) of 52 patients in the non-irradiated group survived their relapse, with a median follow-up after relapse of 7.56 years (6.15–15.40 appendix p 8). Consistent with univariable survival analyses (appendix p 7), desmoplastic/nodular histology was the most frequently observed histological subtype in survivors in this group (seven [64%] of 11 patients); most survivors received craniospinal irradiation (eight [73%] of 11 patients) and had re-resection

Figure 2: Time to relapse and pattern of relapse according to molecular subgroup in the irradiated group

(A) Time to relapse in the four consensus molecular subgroups in patients who received upfront CSI (irradiated group). (B) Schematic representing the patients with distant disease at diagnosis, emergent distant disease at relapse, and distant disease at relapse according to molecular subgroup. (C) Different patterns of relapsed disease according to molecular subgroup and relapse pattern. MB_{SHH}=19 patients, MB_{Group3}=25 patients, MB_{Group4}=58 patients. CSI=craniospinal irradiation. MB=medulloblastoma.

(six [60%] of ten patients with available data) at relapse. The seven survivors with tumours with desmoplastic/nodular histology were MB_{SHH} (MB_{SHH-DN}; appendix p 8). Overall, seven [64%] of 11 patients with MB_{SHH-DN} tumours survived their relapse. Of these seven survivors with MB_{SHH-DN} tumours, all received radiotherapy at relapse (five received craniospinal irradiation and two received focal irradiation; appendix p 8). Of note, of the four patients with MB_{SHH-DN} who died, two had data on their treatment history available; both patients did not receive any radiotherapy at relapse.

Patterns of disease relapse were also molecular subgroup dependent in the irradiated group. Isolated local relapses were most commonly observed in patients with MB_{SHH} tumours (seven [35%] of 20 relapses; $p=0.0028$); whereas, patients with MB_{Group3} and MB_{Group4} tumours had predominantly distant relapses (figure 2B). Although distant relapses were also predominant in patients with MB_{SHH} tumours (13 [65%] of 20 relapses), these were at a significantly lower frequency than in patients with MB_{Group3} tumours (23 [92%] of 25) and MB_{Group4} tumours (56 [90%] of 62; $p=0.011$; figure 2B). However, rates of emergence of distant disease at relapse in the irradiated group did not differ between molecular subgroups ($p=0.19$; figure 2B). Approximately 80% of all patients with local-only disease at diagnosis displayed emergent distant disease at relapse (figure 2B).

Of the 20 isolated local relapses, 19 were significantly associated with nodular disease ($p<0.0001$; appendix p 5). However, although patients who locally relapsed with MB_{SHH} tumours were significantly associated with nodular disease ($p=0.016$; appendix p 5) this finding was not true for patients who locally relapsed with MB_{Group3} and MB_{Group4} tumours, because of their differing patterns of distant relapse. Patients with MB_{SHH}, MB_{Group3}, and MB_{Group4} tumours all had distant-diffuse relapses (figure 2C). However, distant nodular relapses were almost exclusively observed in patients with MB_{Group3} and MB_{Group4} tumours (figure 2C). Furthermore, in post-hoc exploratory analyses, nine (82%) of 11 patients with distantly relapsed diffuse MB_{SHH} tumours had *MYCN* amplification at diagnosis, compared with two [25%] of eight patients with nodular MB_{SHH} relapses ($p=0.024$; appendix p 5). Distant diffuse patterns were associated with early relapses (within 18 months of initial diagnosis) in patients in the irradiated group with MB_{Group3} tumours ($p=0.0048$; appendix p 5), this association was not observed in patients with MB_{SHH} or MB_{Group4} tumours.

Subgroup-specific patterns of relapse were also observed in the non-irradiated group. Patients with MB_{SHH} ($p<0.0001$) and MB_{Group3} ($p=0.060$) tumours acquired distant relapses (appendix p 9) and had predominantly distant disease at relapse (17 [71%] of 24 MB_{SHH} tumours and 12 [80%] of 15 MB_{Group3} tumours; appendix p 9). MB_{SHH} and MB_{Group3} displayed similar proportions of distant-diffuse and distant-nodular relapses ($p=0.71$; appendix p 9); local-nodular relapses

	Frequency	Univariable analyses		Multivariable analyses	
		HR (95% CI)	p value	HR (95% CI)	p value
Clinicopathological features and treatment at diagnosis					
Boys	133/178 (75%)	0.98 (0.69–1.38)	0.90
Subtotal resection	58/178 (33%)	0.92 (0.67–1.27)	0.84
Chemotherapy	142/177 (80%)	1.06 (0.72–1.54)	0.83
Classic histology	123/162 (76%)	0.83 (0.58–1.19)	0.71
Large-cell anaplastic histology	26/162 (16%)	1.42 (0.93–2.18)	0.43
Desmoplastic/ nodular histology	13/162 (8%)	0.89 (0.50–1.58)	0.79
Distant disease	57/170 (34%)	1.29 (0.93–1.78)	0.39
Established molecular features at diagnosis					
MYC amplification	5/157 (3%)	3.13 (1.27–7.68)	0.069	16.77 (5.37–52.45)	<0.0001
MYCN amplification	20/155 (13%)	1.28 (0.80–2.05)	0.63
TP53 mutation	18/150 (12%)	1.40 (0.85–2.31)	0.51
Isochromosome 17q	40/117 (34%)	0.87 (0.59–1.28)	0.78
TERT mutation	10/162 (6%)	0.76 (0.40–1.46)	0.73
MB _{WNT}	5/156 (3%)	0.75 (0.31–1.84)	0.77
MB _{SHH}	29/156 (19%)	1.10 (0.73–1.65)	0.79
MB _{Group3}	40/156 (26%)	1.88 (1.30–2.72)	0.012	1.77 (1.21–2.59)	0.0031
MB _{Group4}	82/156 (53%)	0.65 (0.47–0.89)	0.063
HR=hazard ratio. MB=medulloblastoma.					
Table 3: Univariable and multivariable analyses of correlates of time to relapse in the irradiated group					

were more commonly observed in patients with MB_{SHH} tumours (appendix p 9).

Exploratory analyses of second-generation molecular subtypes within MB_{Group3} and MB_{Group4} in the irradiated group showed further insights into the patterns and timings of relapse. Analyses of MB_{Group3} and MB_{Group4} subtypes I–VIII (figure 3A) in 90 patients revealed a differing disease course between subtypes (time to relapse $p<0.0001$; time from relapse to death $p=0.00012$; and overall survival $p=0.0086$; figure 3B; appendix p 10). For example, group II, associated with MB_{Group3} and *MYC* amplification, showed the shortest time to death after relapse (median 0.26 years [0.09–0.55], $p=0.0063$; appendix p 11). Similar to the consensus MB_{Group3} and MB_{Group4} molecular subgroups, subtypes II ($p=0.0098$), III ($p=0.0046$), and VIII ($p<0.0001$) developed distant disease at relapse whereas subtypes V ($p=0.32$) and VII ($p=0.16$) maintained their distant disease status between diagnosis and relapse (table 4). However, patterns of distant disease at relapse did not differ significantly between subtypes (figure 3C).

Evaluating the methylation subtypes described by Schwalbe and colleagues,² MB_{Group3-HR} had a rapid disease course (time to relapse $p<0.0001$; time from relapse to death $p=0.0011$; overall survival $p<0.0001$; appendix p 12). Rates of emergent distant disease at relapse were similar across subtypes (appendix p 12). In multivariable analyses of the irradiated MB_{Group3} and MB_{Group4} tumours, MB_{Group4-HR} had increased time to relapse whereas subtype III had decreased time to relapse (appendix p 11). Subtype II was

associated with a rapid disease course after relapse (appendix p 11), whereas nodular disease at relapse was associated with a prolonged time from relapse to death (HR 0.42, 0.21–0.81). Finally, MB_{Group3-HR} and

MYC amplification were associated adversely with overall survival (appendix p 11).

Discussion

We have presented the largest study of the nature and correlates of disease relapse in patients with medulloblastoma. Our results suggest that treatment and molecular subgroup are associated with the nature of relapse and subsequent disease course. These findings can help to inform disease management through improved post-therapy surveillance and post-relapse prognostication.

In the irradiated group, disease course varies according to molecular subgroup; patients with MB_{Group4} tumours were associated with a prolonged time to relapse (appendix p 14) and consequently warrant a prolonged surveillance period of up to 10 years. Furthermore, patterns of relapse were molecular subgroup dependent. Local-nodular relapses were more common in MB_{SHH} tumours as previously reported.⁵ However, MB_{SHH}, MB_{Group3}, and MB_{Group4} molecular subgroups all developed a significant percentage of distant relapses. Of note, this finding does not support suggestions by others to intensify the local radiotherapy boost delivered to patients with MB_{SHH} tumours at diagnosis to reduce relapse rates; isolated local MB_{SHH} relapses in our cohort were in the minority.⁵

Our study details the subgroup specific patterns of distant relapse that can aid both disease surveillance and post-relapse prognostication (appendix p 14). Following craniospinal irradiation, distantly relapsed MB_{SHH} almost exclusively displayed diffuse disease, which is associated with MYCN amplification at diagnosis. Therefore, MRI surveillance after initial treatment of patients with MYCN amplified MB_{SHH} should always consider the whole neuroaxis, even if the patient had local-only disease at diagnosis. Distantly relapsed MB_{Group3} and MB_{Group4} tumours displayed both nodular and diffuse patterns of disease. Nodular relapse was associated with prolonged survival post-relapse in patients with MB_{Group3} and MB_{Group4} tumours. Together, these findings should be used to inform clinical discussions and treatment decisions. For example, nodular disease is more amenable to local therapy strategies (re-resection and focal irradiation), which might be justified given this new evidence of their prolonged survival after relapse.

Patients not treated with upfront craniospinal irradiation relapsed quickly, with more than half of all children relapsing within 18 months of initial diagnosis.

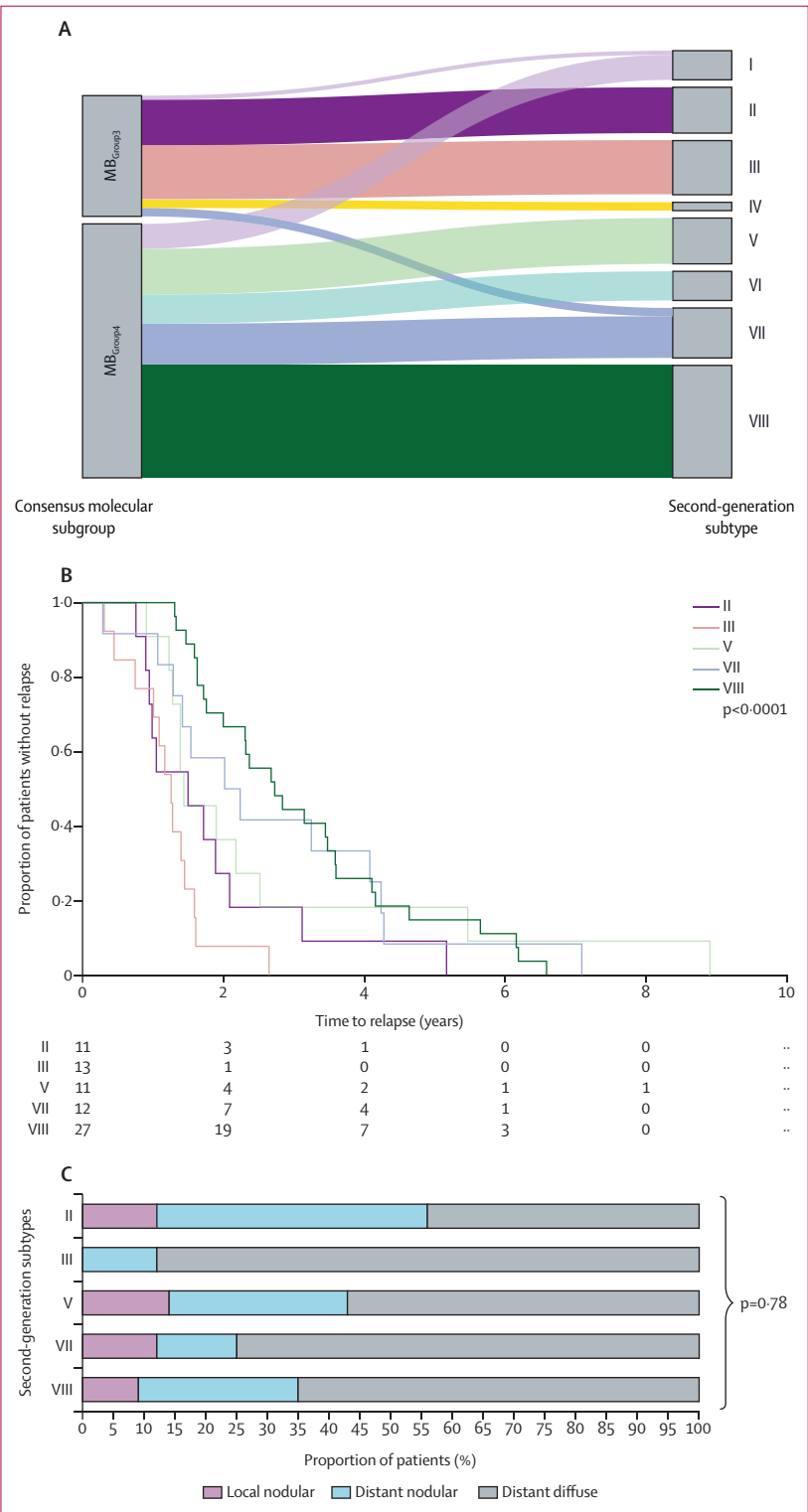


Figure 3: Assessment of relapse characteristics by second-generation MB_{Group3} and MB_{Group4} subtypes
(A) Sankey plot of the relationship between consensus MB_{Group3} and MB_{Group4} and second-generation subtypes I–VIII. (B) Time to relapse according to second-generation MB_{Group3} and MB_{Group4} molecular subtype. (C) The different patterns of relapsed disease according to second-generation MB_{Group3} and MB_{Group4} molecular subtype. Subtype II=9 patients. Subtype III=8 patients. Subtype V=7 patients. Subtype VII=8 patients. Subtype VIII=22 patients. MB=medulloblastoma.

	Distant disease at diagnosis*	Distant disease at relapse†	p value‡	Acquired distant disease§
II	3/11 (27%)	8/9 (89%)	0.0098	6/7 (86%)
III	4/13 (31%)	8/8 (100%)	0.0046	7/7 (100%)
V	6/11 (55%)	6/7 (86%)	0.32	3/4 (75%)
VII	6/12 (50%)	7/8 (87%)	0.16	2/3 (67%)
VIII	6/27 (22%)	22/24 (92%)	<0.0001	17/19 (89%)

Only MB_{Group3} and MB_{Group4} subtypes present in >10% of cohort included in analyses (I, IV, and VI excluded from analyses). MB=medulloblastoma. *No significant difference by molecular subtype p=0.25. †No significant difference by molecular subtype p=0.88. ‡p value comparing the proportion of distant disease at diagnosis versus distant disease at relapse within MB_{Group3} and MB_{Group4} second-generation subtypes. §No significant difference by molecular subtype p=0.58.

Table 4: The prevalence of distant disease at diagnosis and distant disease acquired at relapse according to second-generation MB_{Group3} and MB_{Group4} molecular subtype

This rapid disease course is compounded by the presence of recognised high-risk features (eg, *MYC* amplification and large-cell anaplastic histology) that inform current clinical management. However, patterns of distantly-relapsed non-irradiated MB_{SHH} and MB_{Group3} tumours differed to those observed in the irradiated group and were not associated with disease course after relapse. Treatment at relapse (eg, craniospinal irradiation at relapse and re-resection), MB_{SHH}, and desmoplastic/nodular histology were associated with a more favourable disease course after relapse. Together, these findings inform counselling at diagnosis and relapse, identifying a subcohort of patients with potentially salvageable disease should it relapse (appendix p 14).

Finally, analyses of novel second-generation molecular subtypes suggest specific subtypes further resolve the heterogeneous nature of MB_{Group3} and MB_{Group4} relapses after treatment with craniospinal irradiation. For example subtype II tumours displayed a rapid time to death after relapse. These findings emphasise the importance of understanding tumour biology at diagnosis, how this relates to the nature of disease relapse and underlying mechanisms, and, in the future, how these could influence prognostication after relapse.

However, this study does have some limitations. Although the study is retrospective and is not based on a uniformly treated clinical trial cohort, all patients were managed using current contemporary treatment strategies appropriate to their age. Of note, we considered the retrospective nature of this cohort in our assessments; no variable or outcome measure was biased by treatment era (before 2000 vs 2000 and after). To allow us to answer the study questions, only patients who relapsed with disease were selected for this study. Overall, we estimate our cohort comprises approximately 50% of all patients (aged up to 18 years) in the UK with medulloblastoma relapse during the study collection period. By contrast to all previous studies,^{5,7} we have considered upfront-treatment differences, and analysed the patterns and timings of relapse in conjunction with detailed annotation of all

established disease-wide features at diagnosis and novel second-generation methylation subtypes.

In summary, consideration of upfront diagnostic disease features, coupled with time and pattern of relapse, offers opportunities to improve the clinical management of medulloblastoma.

Contributors

RMH, SB, and SCC conceived of and designed the study. RMH, SR, DH, JCL, and SC did the laboratory experimentation and analysis. RMH and ECS did the statistical analysis. RMH, ECS, GR, and DW did the bioinformatics analysis. RMH, ECS, and YG prepared the figures. SBW, TSJ, and AJ did the central pathological review. RMH, SBW, TSJ, AM, AJ, BP, and SB provided patient data and clinical interpretation. All authors are accountable for all aspects of the work and approved the final copy.

Declaration of interests

TSJ reports grants from the National Institute of Health Research during the study; grants from Cancer Research UK and Olivia Hodson Cancer Fund outside the submitted work; and is a director and shareholder of Repath and Neuropath. All other authors declare no competing interests.

Acknowledgments

This study was funded by Cancer Research UK, Action Medical Research, The Tom Grahame Trust, The JGW Patterson Foundation, Star for Harris, The Institute of Child Health - Newcastle University - Institute of Child Health High-Risk Childhood Brain Tumour Network (co-funded by The Brain Tumour Charity, Great Ormond Street Children's Charity, and Children with Cancer UK).

References

- Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization classification of tumors of the central nervous system: a summary. *Acta Neuropathologica* 2016; **131**: 803–20.
- Schwalbe EC, Lindey JC, Nakjang S, et al. Novel molecular subgroups for clinical classification and outcome prediction in childhood medulloblastoma: a cohort study. *Lancet Oncol* 2017; **18**: 958–71.
- Sharma T, Schwalbe EC, Williamson D, et al. Second-generation molecular subgrouping of medulloblastoma: an international meta-analysis of group 3 and group 4 subtypes. *Acta Neuropathologica* 2019; **138**: 309–26.
- Hill RM, Kuijper S, Lindsey JC, et al. Combined *MYC* and *P53* defects emerge at medulloblastoma relapse and define rapidly progressive, therapeutically targetable disease. *Cancer Cell* 2015 **27**: 72–84.
- Ramaswamy V, Remke M, Bouffet E, et al. Recurrence patterns across medulloblastoma subgroups: an integrated clinical and molecular analysis. *Lancet Oncol* 2013; **14**: 1200–07.
- Pizer B, Donachie PHJ, Robinson K, et al. Treatment of recurrent central nervous system primitive neuroectodermal tumours in children and adolescents: results of a Children's Cancer and Leukaemia Group study. *Eur J Cancer* 2011; **47**: 1389–97.
- Sabel M, Fleischhack G, Tippelt S, et al. Relapse patterns and outcome after relapse in standard risk medulloblastoma: a report from the HIT-SIOP-PNET4 study. *J Neurooncol* 2016; **129**: 515–24.
- Muller K, Mynarek M, Zwiener I, et al. Postponed is not canceled: role of craniospinal radiation therapy in the management of recurrent infant medulloblastoma—an experience from the HIT-REZ 1997 & 2005 studies. *Int J Radiat Oncol Biol Phys* 2014; **88**: 1019–24.
- Rutkowski S, Gerber NU, von Hoff K, et al. Treatment of early childhood medulloblastoma by postoperative chemotherapy and deferred radiotherapy. *Neuro Oncol* 2009; **11**: 201–10.
- Aguilera D, Mazewski C, Fangusaro J, et al. Response to bevacizumab, irinotecan, and temozolomide in children with relapsed medulloblastoma: a multi-institutional experience. *Childs Nerv Syst* 2013; **29**: 589–96.
- Wetmore C, Herington D, Lin T, et al. Reirradiation of recurrent medulloblastoma: does clinical benefit outweigh risk for toxicity? *Cancer* 2014; **120**: 3731–37.
- Sterba J, Pavelka Z, Andre N, et al. Second complete remission of relapsed medulloblastoma induced by metronomic chemotherapy. *Pediatric Blood Cancer* 2010; **54**: 616–17.

- 13 Peyrl A, Chocholous M, Kieran MW, et al. Antiangiogenic metronomic therapy for children with recurrent embryonal brain tumors. *Pediatric Blood Cancer* 2012; **59**: 511–17.
- 14 Northcott PA, Robinson GW, Kratz CP, et al. Medulloblastoma. *Nat Rev Dis Primers* 2019; **5**: 11.
- 15 Chang CH, Housepian EM, Herbert C Jr. An operative staging system and a megavoltage radiotherapeutic technic for cerebellar medulloblastomas. *Radiology* 1969; **93**: 1351–59.
- 16 Dufour C, Beaugrand A, Pizer B, et al. Metastatic medulloblastoma in childhood: Chang's classification revisited. *Int J Surg Oncol* 2012; **2012**: 245385.
- 17 Ramaswamy V, Remke M, Bouffet E, et al. Risk stratification of childhood medulloblastoma in the molecular era: the current consensus. *Acta Neuropathologica* 2016; **131**: 821–31.
- 18 Shih DJ, et al. Cytogenetic prognostication within medulloblastoma subgroups. *J Clin Oncol* 2014; **32**: 886–96.
- 19 Remke M, Ramaswamy V, Peacock J, et al. TERT promoter mutations are highly recurrent in SHH subgroup medulloblastoma. *Acta Neuropathologica* 2013; **126**: 917–29.
- 20 Lindsey JC, et al. TERT promoter mutation and aberrant hypermethylation are associated with elevated expression in medulloblastoma and characterise the majority of non-infant SHH subgroup tumours. *Acta Neuropathologica* 2014; **127**: 307–09.
- 21 Schwalbe EC, Williamson D, Lindsey JC, et al. DNA methylation profiling of medulloblastoma allows robust subclassification and improved outcome prediction using formalin-fixed biopsies. *Acta Neuropathologica* 2013; **125**: 359–71.
- 22 Schwalbe EC, Hicks D, Rafiee G, et al. Minimal methylation classifier (MIMIC): a novel method for derivation and rapid diagnostic detection of disease-associated DNA methylation signatures. *Sci Rep* 2017; **7**: 13421.
- 23 Robinson GW, Rudneva VA, Buchhalter I, et al. Risk-adapted therapy for young children with medulloblastoma (SJYC07): therapeutic and molecular outcomes from a multicentre, phase 2 trial. *Lancet Oncol* 2018; **19**: 768–84.